Finding Genes by Leaps and Bounds

HAVING completed the sequencing of the human genome—as well as those of other vertebrates such as the mouse and the chicken—researchers in functional genomics are now trying to understand exactly when and where during human development individual genes turn on or off and what exactly they do.

To answer these questions, researchers at Lawrence Livermore are using computational methods to compare the genomes of different species to find common gene sequences (strings of nucleotides and the building blocks of DNA). Although it may seem that looking to frogs and mice for answers to questions about the human genome is a leap, it is not a very long one. Biologists reason that if a gene (and its function) is the same—or has been "conserved"—between a frog and a mouse, whose last common ancestor lived 340 million years ago, chances are very good that the gene will also be conserved between the mouse and the human, whose last common ancestor lived only 75 million years ago.

According to Gabriela Loots, in the Genome Biology Division of the Biosciences Directorate, the thought is that if evolution has selected the conservation of these genes from species to species, they probably confer some important, basic developmental function in the vertebrate embryo, such as tissue differentiation or organ specification. "All vertebrate embryos go through similar development during the early stages of embryogenesis," says Loots. "Whereas each vertebrate species eventually develops its own identity, many similarities exist across different groups of species throughout all developmental stages. It is fair to assume that similar proteins perform similar biological functions."

Scientists reason that if a gene sequence is conserved between species residing at extremes of the evolutionary tree, for example humans and fishes, those gene sequences likely share an ancestral biological function. By identifying these regions of commonality, biologists can home in on those genes that perform critical functions during the early development of human embryos.

Understanding which genes trigger which biological processes can help scientists pinpoint gene defects that cause disease. Researchers hope to use this knowledge to develop diagnostic tests and predictive tools as well as new drugs, therapies, and interventions for both genetic and acquired diseases. In addition, the gathered information will broaden understanding of how complex genomes are organized and how genes cooperate with one another to initiate cascades of regulatory networks.

Xenopus tropicalis (shown) is a much better choice for functional genomic studies than the traditionally used laboratory frog *Xenopus laevis*.

Mammalian genomes encode for an estimated 25,000 unique genes. The process of elucidating the function for each transcript has been lagging behind scientists' ability to sequence large vertebrate genomes. So far, less than 50 percent of all known genes in the human genome have been experimentally tested to decipher how they function in a living organism. Enormous resources have been invested in cataloging expressed sequence tags (a stretch of coding DNA that has been derived from RNA) to determine the full-length messenger RNA (mRNA) sequences for the most abundantly transcribed vertebrate genes. However, the process of determining the exact biological role of each transcript in a living organism has been slow, and researchers are still far from linking all known gene sequences to what they do in vivo. Livermore scientists are developing novel high-throughput methods to study how individual genes function during embryonic development in living organisms.

So, What About the Frogs?

Loots's group is using a tropical frog, *Xenopus tropicalis*, to create a model that allows controlled gene expression and can be monitored in vivo. Funded in part by Livermore's Laboratory Directed Research and Development Program, this frog model will help in identifying and characterizing the biological functions of some of the evolutionarily conserved genes. The Department of Energy's Joint Genome Institute in Walnut Creek, California, is sequencing the *X. tropicalis* genome. This work is expected to be completed by summer 2005.

The benefits of using frogs instead of mice for this research are compelling. One of the most obvious benefits is that researchers can watch a frog embryo change through various stages of early development in a petri dish. Because frogs have large eggs (over 1 millimeter in diameter), the resulting embryos are easily viewed under a standard dissecting microscope. Also, *X. tropicalis* females can be induced to lay eggs "on command" at any time of the year. After inducing female frogs with hormones, the researchers harvest their eggs by gently squeezing them. The eggs are then fertilized in a petri dish.

Another key advantage is that *X. tropicalis* is the only amphibian that is diploid. That is, it has two sets of chromosomes rather than the usual four sets (tetraploidy) possessed by all other frogs, including the classic laboratory frog *Xenopus laevis*. Because *X. tropicalis* has half the genome of *X. laevis*, it is an ideal subject for silencing gene expression (and, subsequently, gene function) in laboratory experiments. Loots and her group use synthesized strands of genetic material called morpholino oligonucleotides (MOs) to perform the loss-of-function experiments in the frog embryos. These MOs are single-stranded sequences that target the gene of interest and silence that gene before it has a chance to initiate protein production.

Step by Step

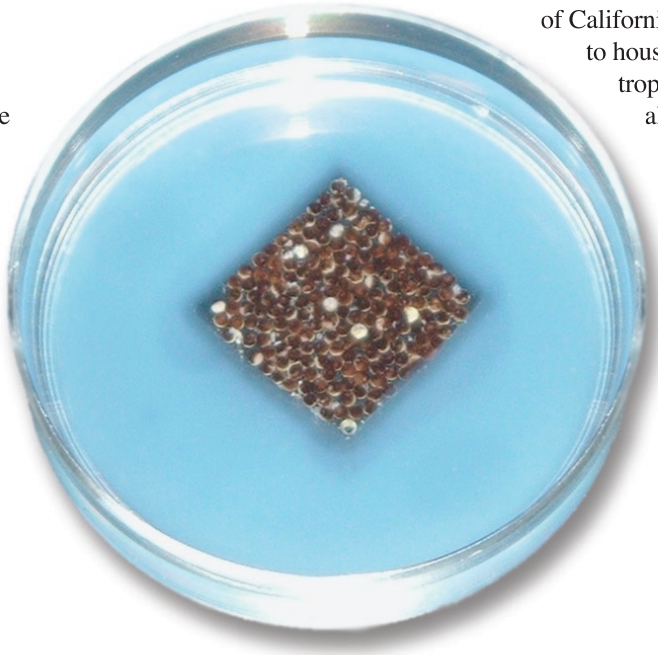
Before any work is done in the laboratory, researchers carry out comparative sequence analyses between all available vertebrate genomes. These analyses emphasize similarities between the frog and the human genomes using computational methods developed by bioinformatician Ivan Ovcharenko. From the resulting data, regions of high conservation are identified and a gene set of interest is defined.

To validate this gene set, the main task is to determine when and where each gene is turned on. This task is accomplished by making a complementary copy of the gene's mRNA sequence that is labeled with a marker. The resulting sequence is called the anti-sense probe. This probe is used to detect where the target gene's mRNA is present, a process by which the probe finds its exact complementary match and attaches, or hybridizes, to it. Then, a color reaction is used to locate where the probe binds to RNA. These locations are imaged and cataloged into a database. Loots's group has refined this approach to permit high throughput: They can detect the embryonic expression of hundreds of different genes in one reaction using a robotic system that expedites the process.

Loots's group uses MOs to silence specific genes in the *X. tropicalis* eggs and then studies the visible genetic traits and the morphological changes in form and structure of the resulting embryos at different stages of development. Similarly, the group evaluates the effects of over-expressing a gene by injecting mRNA for these genes into the eggs.

Ultimately, researchers hope to identify genes that are important during early development, when too much or too little of a gene product can dramatically affect normal biological processes. In particular, Loots's group will be investigating novel genes that specialize in patterning the vertebrate skeletal and muscular structures. "If we can say 'here's a group of genes that are all expressed in the eye,' then that opens up all sorts of opportunities for collaboration and discovery," says Loots. "And if a particular gene functions the same in a frog and a mouse, we can postulate that the gene will likely work the same in humans."

Loots and her colleagues are working with fixed frog embryos that are transferred as needed from Richard Harland's laboratory in the Molecular and Cell Biology Department at the University of California at Berkeley (UCB). However, Loots plans to house a self-sustaining colony of these aquatic, tropical frogs in a facility at Livermore that is already fitted with a filtering warm-water tank system. Frogs from UCB and other licensed vendors will be transferred to the new facility once it has been approved by California's Department of Fish and Game. Loots is eager to ramp up the pace. "Getting the new frog facility up and running will streamline the

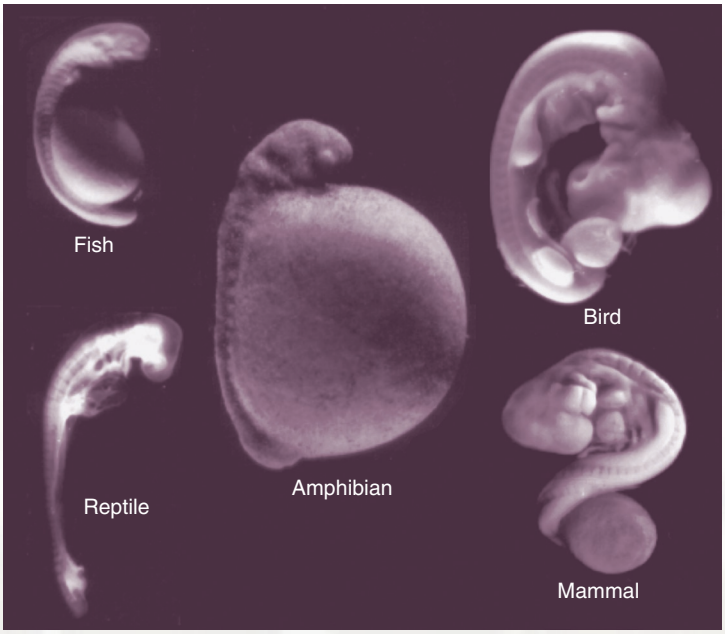


The eggs harvested from *Xenopus tropicalis* are easy to view under a standard dissecting microscope because of their large size (1 to 1.2 millimeters in diameter).

X. tropicalis project and will facilitate novel high-throughput research using this animal model as a vector for in vivo experimentation," says Loots.

One Man's Junk . . .

In an interesting biological twist, it appears that many genes are controlled by important regulatory elements that lie in regions previously considered "junk" DNA because these sequences did not code for proteins. However, scientists now realize that some of these noncoding sequences are also highly conserved across multiple species. Thus, the notion that a group of these DNA segments are junk is being scrapped. Instead, researchers posit that the sequences have an important function in acting on aspects of biology other than encoding for proteins. The current hypothesis is that a significant fraction of these highly conserved, noncoding stretches of DNA might play a critical regulatory role in gene expression, or in turning on and turning off neighboring genes that do code for proteins.



This photograph shows the morphological similarities in embryogenesis across vertebrate species at similar stages of development.

Developing *X. tropicalis* as a high-throughput model for in vivo experimentation expands the horizons in genome biology research. Once a thorough catalog of gene expression has been obtained and the function of each transcript understood, the next major challenge will be to link each transcriptional regulatory element to the gene it regulates. Loots is looking forward to exploiting this novel model organism and leaping into the future of vertebrate genome research.

—Maurina S. Sherman

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